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Virus-producing cell lines

The virus-producing cell line is generated by transfecting pG-MtkPL into the ψ -2 packaging cell line. Virus was prepared from the filtered culture medium of transfected ψ -2 cell lines. NIH3T3 fibroblasts were infected by G-MtkPL, and clones were isolated in a Phleomycin-containing medium.

RESULTS

To assay for I-Sce I endonuclease activity in mammalian cells, NIH3T3 cells containing the G-MtkPL provirus were used. The G-MtkPL provirus (Fig. 25a) contains the tk gene (in place of the gag, pol and env viral genes), for negative selection in gancyclovir-containing medium and, in the two LTRs, an I-Sce I recognition site and the PhleoLacZ fusion gene. The PhleoLacZ gene can be used for positive selection of transduced cells in phleomycine-containing medium.

We hypothesized that the expression of I-Sce I endonuclease in these cells would induce double-strand breaks (DSB) at the I-Sce I recognition sites that would be repaired by one of the following mechanisms (illustrated in Fig. 25): a) if the I-Sce I endonuclease induces a cut in only one of the two LTRs (Fig. 25-b 1 and 2), sequences that are homologous between the two LTRs could pair and recombine leading to an intra-chromosomal homologous recombination (i.e. by single strand annealing (SSA) (12B, 10B) or crossing-over); b) If the I-Sce I endonuclease induces a cut in each of the two LTRs, the two free ends can religate (end joining mechanism (31B) leading to an intra-chromosomal recombination (Fig. 25-b 3); or alternatively c)